PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 May 1998 (25.05.98)	
International application No. PCT/US97/17669	**Applicant's or agent's file reference** 3123-4000PC
International filing date (day/month/year) 30 September 1997 (30.09.97)	**Priority date** (day/month/year) 10 October 1996 (10.10.96)
Applicant EMALFARB, Mark, Aaron et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 April 1998 (30.04.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

A. Addae-Ruesch

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
3123-4000PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 97/ 17669	30/09/1997	10/10/1996
Applicant		
EMALFARB, Mark, Aaron et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant
   - [X] the text has been established by this Authority to read as follows:

   CHRYSOSPORIUM CELLULASE AND METHODS OF USE

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. NONE
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/17669

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/56 C12N9/42 D06M16/00 C12N1/14 C11D3/386
C07K14/37 D21C9/00 C12N15/01 //(C12N9/42,C12R1:645)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N D06M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1998	26/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lejeune, R

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 106 989 A (KOMURA ICHIRO ET AL) 15 August 1978 see abstract see figure 1 see figure 2 see column 1, line 66 - column 2, line 19	2-5,9, 11,12, 16, 18-32, 40-44, 46-50, 54,63, 67,73-80
A		6-8,10, 13-15, 17,45, 51-53, 55-62, 64-66, 68-72
X	WO 95 02675 A (NOVONORDISK AS ;PROCTER & GAMBLE (US); SCHUELEIN MARTIN (DK); CONV) 26 January 1995 see page 11, line 6 - page 15, line 6	33-39
X	EP 0 468 464 A (SHOWA DENKO KK) 29 January 1992 see abstract see page 12; figure 1	60-62
X	UZCATEGUI E. ET AL.: "The 1,4-(beta)-D-glucan glucanohydrolases from Phanerochaete chrysosporium. Re-assessment of their significance in cellulose degradation." JOURNAL OF BIOTECHNOLOGY, vol. 21, no. 1-2, 1991, pages 143-160, XP002055448 see abstract	60,61
A		55-59
P,X	WO 97 14804 A (PRIMALCO LTD ;MIETTINEN OINONEN ARJA (FI); LONDESBOROUGH JOHN (FI)) 24 April 1997 see abstract see figures 1A,1B see figures 5A,5B see page 13, line 10 see page 13, line 4 see page 66 - page 71 see page 84 - page 88	2-5,9, 11,12, 16, 18-50, 54,63, 67,73-80

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ERIKSSON K.-E. & PETTERSSON B.: "Extracellular enzyme system utilized by the fungus Sporotrichum pulverulentum (Chrysosporium lignorum) for the breakdown of cellulose. 1. Separation, purification and physico-chemical characterization of five endo-1,4-(beta)-glucanases." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 51, 1975, pages 193-206, XP002055449 see abstract see page 203; tables 4,5 see page 194, column 1, paragraph 7 ---	55-58
A	ERIKSSON K.-E. & PETTERSSON B.: "Extracellular enzyme system utilized by the fungus Sporotrichum pulverulentum (Chrysosporium lignorum) for the breakdown of cellulose. 3. Purification and physico-chemical characteirsation of an exo-1,4-(beta)-glucanase." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 51, 1975, pages 213-218, XP002055565 see abstract -----	59

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/17669

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4106989 A	15-08-78	JP 1077134 C	25-12-81
		JP 52134090 A	09-11-77
		JP 56022275 B	23-05-81
WO 9502675 A	26-01-95	AU 7069294 A	13-02-95
		BR 9407066 A	12-03-96
		CN 1129011 A	14-08-96
		CZ 9600110 A	12-06-96
		EP 0708819 A	01-05-96
		FI 960132 A	11-03-96
		JP 9500667 T	21-01-97
EP 0468464 A	29-01-92	JP 4079882 A	13-03-92
		CA 2047613 A	25-01-92
		DE 69122687 D	21-11-96
		DE 69122687 T	30-10-97
		US 5314637 A	24-05-94
		US 5318905 A	07-06-94
		US 5231022 A	27-07-93
WO 9714804 A	24-04-97	AU 7299796 A	07-05-97

PATENT COOPERATION TY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MOROZ, Eugene
Morgan & Finnegan, L.L.P.
345 Park Avenue
New York, NY 10154
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	22.01.99
Applicant's or agent's file reference 3123-4000PC	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/17669	30/09/1997	10/10/1996

Applicant
EMALFARB, Mark, Aaron et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Vullo, C Tel. (+49-89) 2399-8061

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
3123-4000PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/17669	30/09/1997	10/10/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/56		
Applicant		
EMALFARB, Mark, Aaron et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 21 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/04/1998	22.01.99
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Ury, A Telephone No. (+49-89) 2399-8411

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/17669

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13,15-26,28,29, 31-34,36,39-75	as originally filed			
14,27,30,35,37, 38	as received on	01/05/1998	with letter of	29/04/1998

Claims, No.:

1-6,8-94	as received on	25/05/1998	with letter of	19/05/1998
7,95	as received on	16/11/1998	with letter of	12/11/1998

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-3, 5-7, 11-63, 65, 66, 68-70, 72-91, 93-95
	No:	Claims	4, 8-10, 64, 67, 71, 92
Inventive step (IS)	Yes:	Claims	1, 5-7, 11-21, 30-35, 40-57, 66, 70, 72, 87
	No:	Claims	2, 3, 22-29, 36-39, 58-63, 65, 68-69, 73-79, 80-86, 88-91, 93-95
Industrial applicability (IA)	Yes:	Claims	1-95
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Item V.

Reference is made to the following document:

D1: US 4,106,989.
"ATCC Filamentous Fungi 19th edition, 1996".
"ATCC Names of Industrial Fungi".

I) 1) Document D1 discloses the strain Sporotrichum cellulophilum AJ 6986 deposited under deposition No. ATCC 20494.
In "ATCC Filamentous Fungi 19th edition, 1996", page 311, Sporotrichum cellulophilum AJ 6986 corresponds to Myceliophthora thermophila.
In "ATCC Names of Industrial Fungi", page 183, Myceliophthora thermophila is a synonym of Chrysosporium thermophilum.
Thus, Sporotrichum cellulophilum AJ 6986 appears to be a specific strain of Chrysosporium thermophilum.

2) D1 discloses that Sporotrichum cellulophilum AJ 6986 (i.e. a Chrysosporium thermophilum strain) produces a thermostable cellulase having cellulase activity at temperature from about 60°C to about 65°C at a pH from about 4.0 to 7.5 (see column 1, line 66 - column 2, line 17 and Figs.1 and 2). In other words, D1 discloses a Chrysosporium thermophilum cellulase having neutral cellulase activity (see the classification according to pH range, page 4, lines 15-21 of the application). A mutant of this strain is also disclosed in D1 (column 3 and claims 4-5).
Thus, D1 destroys the novelty (Article 33.2 PCT) of claims 4, 8-10, 64, 67, 71 and 92 (see Example 1 of D1).

II)1) It is well known that cellulases are produced in fungi (see the description of the application, page 3). Document D1 further shows that a specific specie of Chrysosporium, i.e. Chrysosporium thermophilum (also designated Sporotrichum cellulophilum AJ 6986) produces a neutral cellulase. In order to solve the underlying problem which consists to find other cellulases that are operative at neutral and/or alkaline pH the skilled person would have tried with reasonable expectation of success to find other neutral cellulases in other species of the same genus.

Thus, generic claims such as claims 2, 3, 65, 68-69, 84-86 do not involve an inventive step (Article 33.3 PCT).

2) The uses of neutral cellulases which are either not novel or which do not involve an inventive step (see above) in compositions according to claims 22-29, 36-39, 58-63 and methods according to claims 73-79 are not inventive (Article 33.3 PCT) since similar compositions and methods involving other known neutral cellulases are well known in the art (see for instance "Background of the invention).

III) The method and mutant according to claims 80-83 merely consist in the formulation of obvious desiderata for the skilled person. No inventive step can be recognised for such claims (see e.g. D1, column 2, lines 15-35).

IV) Dependent claims 88-91, 93-95 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step.

V) The claims which are objected to for lack of novelty and/or lack of inventive step seem to fulfil the requirements of Article 33.2 and 3 PCT.

Item VI. WO 97/14804

Item VIII.

It is stressed that the matter for which protection is sought must be defined in terms of the true technical features of the invention, these being not only the features which identify the matter, but also those that allow the skilled person to put the claimed matter into practice without undue experimentation.
Most of the claims contravene Article 6 PCT which states: "the claims shall define the matter for which protection is sought"; which means without addressing the specification.
For instance claims 7, 11-21, 30-35, 40-57, 87 are not acceptable under Article 6 PCT because they relate to products which are merely defined by their activity, their origin (the source fungi defined in a generic form) and one or two parameters (temperature, pH, MW, pI or enzymatic units).

Claims

1. An isolated culture of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D.

2. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a wild type or mutant fungus of the genus *Chrysosporium* in culture in a suitable medium, wherein the fungus is *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, or *Chrysosporium tropicum*.

3. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense*.

4. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

5. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.

6. A composition according to claim 2 wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.

7. A composition according to any one of claims 2-6 having cellulase activity at a temperature from about 40°C to about 60°C, at a pH from about 5.0 to about 11.0.

86. An isolated nucleic acid whose sequence encodes a cellulase enzyme, wherein the cellulase enzyme is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

87. An isolated nucleic acid whose sequence encodes a cellulase according to any one of claims 11-21.

88. A recombinant expression vector comprising a nucleic acid sequence according to any one of claims 85-87.

89. A host cell containing a recombinant expression vector of claim 88.

90. A host cell according to claim 89 wherein the host cell is selected from the group consisting of yeast cells, fungal cells, plant cells, and bacterial cells.

91. A host cell according to claim 90 wherein the host cell is a fungal cell selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Penicillium*, *Chrysosporium*, and *Neurospora*.

92. A method of culturing a fungus of the genus *Chrysosporium*, wherein the fungus is grown in a medium containing inorganic salts, carbon sources, and organic nitrogen sources, at a pH between about 5 and 8.

93. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.5 and 7.5.

94. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.9 and 7.1.

95. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is maintained at 7.5.

Claims

1. An isolated culture of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D.

2. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a wild type or mutant fungus of the genus *Chrysosporium* in culture in a suitable medium, wherein the fungus is *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum,* or *Chrysosporium tropicum.*

3. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense.*

4. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

5. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.

6. A composition according to claim 2 wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.

7. A composition according to any one of claims 2-6 having optimal cellulase activity at a temperature from about 40°C to about 60°C, at a pH from about 5.0 to about 12.0.

AMENDED SHEET

8. A composition according to any one of claims 2-6 having at least 50% of the optimal cellulase activity, at a pH from about 6.0 to about 7.0, at a temperature from about 40°C to about 60°C.

9. A composition according to any one of claims 2-6 wherein said cellulase activity is assayed by any one of the CMCase, RBBCMCase, endoviscometric or filter paper activity assays.

10. A substantially purified and isolated protein fraction, obtained from a composition according to claim 2 or claim 4, and having at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0 as measured by any one of the CMCase, RBBCMCase, endoviscometric or filter paper activity assays.

11. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 25 kD and pI of about 4.

12. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 70 kD and a pI of about 4.

13. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 3.

14. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 43 kD and a pI of about 3.

15. A cellobiohydrolase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 4.

16. A substantially purified and isolated neutral and/or alkaline cellulase enzyme, isolated from a protein fraction according to claim 10, and having a pI of between about 3 and about 5.5.

17. A cellulase according to claim 16 wherein said cellulase possesses either endoglucanase or cellobiohydrolase activity.

18. A cellulase according to claim 16 wherein said cellulase retains at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0.

19. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 25 kD.

20. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 70 kD.

21. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 43 kD.

22. A detergent composition containing one or more purified enzymes isolated from a protein fraction according to claim 10, and further comprising a surfactant.

23. A fabric softening composition containing one or more purified enzymes obtained from the protein fraction according to claim 10.

24. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus

Chrysosporium, said composition having a pH between about 8.0 and about 12.0.

25. The composition of claim 24, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum.*

26. The composition of claim 25, wherein the fungus is *Chrysosporium lucknowense.*

27. A composition according to any one of claims 24-26, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium.*

28. A composition according to any one of claims 24-26, further comprising one or more components selected from the group consisting of pumice stones, abrasives, softeners, solvents, preservatives, bleaching agents, bluing agents, fluorescent dyes, antioxidants, solubilizers, detergents, surfactants, enzymes, builders, anti-redeposition agents, buffers, caking inhibitors, masking agents for factors inhibiting the cellulase activity, and cellulase activators.

29. The composition of claim 28, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium.*

30. The composition of claim 24, wherein the pH is between 10.0 and about 11.0.

31. The composition of claim 25, wherein the pH is between 10.0 and about 11.0.

32. The composition of claim 26, wherein the pH is between 10.0 and about 11.0.

33. The composition of claim 27, wherein the pH is between 10.0 and about 11.0.

34. The composition of claim 28, wherein the pH is between 10.0 and about 11.0.

35. The composition of claim 29, wherein the pH is between 10.0 and about 11.0.

36. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, said composition further comprising one or more components selected from the group consisting of proteinases, detergents, and surfactants.

37. The composition of claim 36, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum.*

38. The composition of claim 37, wherein the fungus is *Chrysosporium lucknowense.*

39. A composition as described in any one of claims 36-38, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

40. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 124 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

41. The composition of claim 40, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

42. The composition of claim 41, wherein the fungus is *Chrysosporium lucknowense*.

43. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 124 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

44. The composition of claim 43, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

45. The composition of claim 44, wherein the fungus is *Chrysosporium lucknowense*.

46. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 191 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

47. The composition of claim 46, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

48. The composition of claim 47, wherein the fungus is *Chrysosporium lucknowense*.

49. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 191 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

50. The composition of claim 49, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

51. The composition of claim 50, wherein the fungus is *Chrysosporium lucknowense*.

52. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 964 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

53. The composition of claim 52, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

54. The composition of claim 54, wherein the fungus is *Chrysosporium lucknowense*.

55. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 964 units of endo-1,4-ß-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

56. The composition of claim 55, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

57. The composition of claim 57, wherein the fungus is *Chrysosporium lucknowense*.

58. A laundry detergent composition, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, further comprising one or more surfactants.

59. The composition of claim 58, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

60. The composition of claim 59, wherein the fungus is *Chrysosporium lucknowense*.

61. A laundry detergent composition, comprising a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, further comprising one or more surfactants.

62. The composition of claim 61, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum, and Chrysosporium tropicum*.

63. The composition of claim 62, wherein the fungus is *Chrysosporium lucknowense*.

64. A cellulase composition having cellulase activity at neutral and/or alkaline pH, obtained from a mutant or wild-type fungus of the genus *Chrysosporium*.

65. A cellulase composition according to claim 64, wherein the fungus is of the species *Chrysosporium lucknowense*.

66. A cellulase composition according to claim 65, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.

67. A method for producing a composition having neutral and/or alkaline cellulase activity, said method comprising growing a wild type or mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

68. The method, according to claim 67, wherein the fungus is *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium queenslandicum,* or *Chrysosporium tropicum*.

69. The method, according to claim 68, wherein the fungus is *Chrysosporium lucknowense*.

70. The method according to claim 69, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500-D.

71. The method according to claim 67, wherein the fungus is a mutant strain of the genus *Chrysosporium*.

72. The method according to claim 71 wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.

73. A method of stonewashing denim fabric or denim jeans, said method comprising treating said denim fabric or denim jeans with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

74. A method of biopolishing, defribillating, bleaching, dyeing, or desizing textiles comprising treating said textiles with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

75. A method of deinking or biobleaching paper or pulp, said method comprising treating said paper or pulp with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

76. A method for enhancing the softness or feel of cellulose or cotton-containing fabric, comprising treating said fabric with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

77. A method according to any one of claims 73-76, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

78. A method according to any one of claims 73-76, wherein the fungus is *Chrysosporium lucknowense*.

79. A method according to claim 77, wherein the fungus is *Chrysosporium lucknowense*.

80. A method for generating mutant strains of the genus *Chrysosporium* which produce enhanced cellulase activity at neutral and/or alkaline pH's, comprising

(a) mutating spores of a fungus of the genus *Chrysosporium*;

(b) culturing the spores from step (a); and

(c) screening the cultures from step (b) for enhanced levels of neutral and/or alkaline cellulase activity.

81. The method of claim 80 wherein the mutating step comprises exposing the spores to ultraviolet light or a chemical mutagen.

82. The method of claim 81 wherein the chemical mutagen is nitrous acid, N-methyl-N'-nitro-N-nitrosoguanidine, or 4-nitroquinolone-N-oxide.

83. A mutant strain of the genus *Chrysosporium* obtained by the method of any one of claims 80-82.

84. A method of isolating genes encoding cellulase enzymes of *Chrysosporium* comprising:

a) isolating a protein from a neutral and/or alkaline cellulase composition produced by a wild type or mutant *Chrysosporium*;

b) sequencing all or part of the protein isolated in step (a);

c) producing a nucleic acid probe derived from the sequence of step (b);

d) screening a wild type or mutant *Chrysosporium* library with the nucleic acid probe of step (c);

e) isolating a nucleic acid sequence recognized by the probe; and

f) sequencing the nucleic acid sequence isolated in step (e).

85. A nucleic acid sequence obtained by the method of claim 84.

86. An isolated nucleic acid whose sequence encodes a cellulase enzyme, wherein the cellulase enzyme is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

87. An isolated nucleic acid whose sequence encodes a cellulase according to any one of claims 11-21.

88. A recombinant expression vector comprising a nucleic acid sequence according to any one of claims 85-87.

89. A host cell containing a recombinant expression vector of claim 88.

90. A host cell according to claim 89 wherein the host cell is selected from the group consisting of yeast cells, fungal cells, plant cells, and bacterial cells.

91. A host cell according to claim 90 wherein the host cell is a fungal cell selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Penicillium*, *Chrysosporium*, and *Neurospora*.

92. A method of culturing a fungus of the genus *Chrysosporium*, wherein the fungus is grown in a medium containing inorganic salts, carbon sources, and organic nitrogen sources, at a pH between about 5 and 8.

93. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.5 and 7.5.

94. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.9 and 7.1.

For example, in ion exchange chromatography, it is possible to separate the cellulase components by eluting with a pH gradient, or a salt gradient, or both. Such separations can be done by those skilled in the art having the benefit of the teachings provided herein.

Once the individual enzymatic components of the cellulase composition are fractionalized and isolated the proteins may be partially sequenced or microsequenced to design synthetic DNA or probes to isolate the gene encoding the enzymatic proteins of interest. Generally the amino terminal sequence of the protein is determined by conventional protein sequencing methods or by automated sequence (Ausubel et al., (1987) in "Current Protocols in Molecular Biology", John Wiley and Sons, New York, New York). Alternatively, other regions of the protein may be sequenced in combination with chemical cleavage or enzymatic cleavage and protein separation techniques. (Ausubel et al., (1987) in "Current Protocols in Molecular Biology", John Wiley and Sons, New York, New York). One of skill in the art will understand that the synthetic DNA clones or probes can be used in routine cloning techniques to isolate the genes corresponding to the enzymes present in the neutral/alkaline cellulase compositions produced by *Chrysosporium*.

It will be understood by one skilled in the art that nucleic acid sequences obtained by this invention in the art may vary due to the degeneracy of the genetic code variations in the DNA sequence, but will still result in a DNA sequence capable of encoding the enzymatic components of the cellulase compositions. Such DNA sequences are therefore functionally equivalent to the nucleic acid sequences of the instant invention and are intended to be encompassed within the present invention. Also intended to be encompassed within this invention are nucleic acid sequences which are complementary to nucleic acid sequences capable of hybridizing to the disclosed nucleic acid sequence under a variety of conditions.

This invention further includes the nucleic acid sequences encoding the enzymes of the cellulase compositions of this invention and

47.18.1 to 47.22.1 were produced by the same manner in shake flasks with Getchinson medium, but containing lactose (0.5 % w/v) and peptone (0.5 % w/v) instead of sweet beet pulp, barley malt and wheat bran. The cell mass was separated by centrifugation and the cell free supernatant was lyophilized and stored for further tests. Preparation #'s 47.1000, 47.1001, 47.2000 & 47.2001 were produced in shake flasks by the same manner as preparation #'s 47.1.1 - 47.15.1 except that they were produced using other *Chrysosporium* strains. Specifically, 47.2001 was produced by *Chrysosporium pannorum*, preparation 47.2000 was produced by *Chrysosporium pruinosum*, preparation 47.1001 was produced by *Chrysosporium keratinophilim* and preparation 47.1000 was produced by *Chrysosporium queenslandicum* (see Example 8). The protein content and activity fingerprints of these C1 preparations are shown in Table 4.

described in Examples 13 and 15. Preparation 47.0325 was produced using a batch fermentation and 47.0528 was produced using a fed batch fermentation protocol.

4. Preparation of Humicola wild type preparation #'s 14.22.1 & 14.23.1 The wild type *Humicola grisea var. thermoidea* preparation # 14.22.1 was produced from the ATCC 16453 strain and the wild type *Humicola insolens* preparation # 14.23.1 was produced from the ATCC 16454 strain. These Humicola wild type preparations were produced in shake flasks using the same method as described above for (Production in shake flasks) of C1 preparation #'s 47.1.1 - # 47.15.1.

Example 6 -- Comparison of C1 to Other Neutral Cellulases

The FPA, CMCase and endoglucanase activities of C1 enzyme preparation # 47.0528 were compared to commercial *Humicola insolens* (Denimax XT) and to wild ATCC-type Humicola (preparation #'s 14.22.1 *Humicola grisea var. thermoidea* (ATCC 16453) & 14.23.1 *Humicola insolens* (ATCC 16454) neutral cellulases. The results are given in the Table 6. The total activities of C-1 # 47.0528 are clearly higher than those of neutral cellulases from wild type *Humicola* and from commercial *Humicola insolens* preparation. The specific CMCase and endoglucanase activities (as units per gram of dry preparation or units per gram of protein) of C-1 47.0528 are higher than those of all tested *Humicola* preparations listed in Table 6. The specific FPA of C-1 # 47.0528 is higher than the specific FPA of Humicola wild type preparations # 14.22.1 & 14.23 and slightly lower than the specific FPA of the *Humicola insolens* commercial product Denimax XT. The pH and thermal stability of C1 cellulase was similar to Denimax XT.

The CMCase of C1 exhibits high stability at optimal pH and temperature: For Example; at pH 7.2 and 50 C CMCase possesses 95 % activity after 1 hour and 75 % activity after 5 hours, at pH 7.7 and 50 C CMCase possesses 93 % activity after 1 hour and 45 % activity after 5 hours (See Table 9.).

Example 8 -- Neutral and or alkaline Cellulase activity/ performance demonstrated in Other Strains of the Same Genera of *Chrysosporium*

Various strains of the *Chrysosporium* genus were tested for cellulase production. The full names and origins of these strains are described below.

Strains obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, include:

1. ATCC 44006 *Chrysosporium lucknowense*
2. ATCC 34151 *Chrysosporium pannorum*
3. ATCC 24782 *Chrysosporium pruinosum*

Strains obtained from the Russian Collection of Microorganisms (VKM) include:

1. VKMF-2119 *Chrysosporium keratinophilum*
2. VKMF-2875 *Chrysosporium keratinophilum*
3. VKMF-2120 *Chrysosporium lobatum*
4. VKMF-2121 *Chrysosporium merdarium*
5. VKMF-2116 *Chrysosporium queenslandicum*
6. VKMF-2117 *Chrysosporium queenslandicum*
7. VKMF-2877 *Chrysosporium tropicum*

Two types of growth media were used in this study: medium A - Getchinson with sugar beet press, barley malt, and wheat bran; and medium B - Getchinson with peptone and lactose. The compositions of the media are described in Table 11.

Table 12. Cellulase production by different strains of *Chrysosporium*

Strains	medium A (6 days)			medium A (7 days)			medium B (5 days)		
	RS	CMCase		RS	CMCase		RS	CMCase	
		pH 5	pH 7		pH 5	pH 7		pH 5	pH 7
1 VKMF 2117	2.7	0	0.46	2.6	0.00	0.00	2.3	0.21	0.09
2. VKMF 2116	1.1	0.22	0.04	0.2	0.38	0.61	4.0	0.58	0.59
3. VKMF 2121	1.9	0	0.57	1.1	0.25	0.10	2.5	0.25	0.09
4. ATCC 24782	3.4	0.33	1.40	1.9	1.85	0.11	3.0	1.10	0.06
5. ATCC 34151	1.0	1.54	0.90	0.9	0.17	0.20	4.3	0.81	0.90
6. ATCC 44006	4.4	0.21	0.49	2.0	0.68	0.34	2.5	1.29	0.06
7. VKMF 2119	4.1	0	0.08	2.7	0.29	0.00	3.8	0.95	0.04
8. VKMF 2120	4.5	0	0.17	2.3	0.23	0.00	2.3	0.12	0.00
9. VKMF 2875	1.6	0	1.01	1.7	0.00	0.00	3.8	1.96	0.05
10. VKMF 2877	2.4	0	0.03	0.8	0.22	0.00	5.0	0.43	0.00
11. C1 (VKMF 3500D)	2.9	1.70	1.65	nt	nt	nt	0.1	0.89	0.80

RS = concentration of reducing sugars in the fermentation medium at the end of fermentation, g/L (Nelson-Somogyi method).
pH 5, pH 7 = the values of pH under which the CMCase activity of the fermentation broth was assayed.
CMCase activity in U/ml.
nt = not tested

In the cases of strains ATCC 34151 *Chrysosporium pannorum*, ATCC 24782 *Chrysosporium pruinosum*, VKMF-2875 *Chrysosporium keratinophilum*, VKMF 2116 *Chrysosporium queenslandicum* the cell mass was separated by centrifugation and cell free supernatant concentrated from 5 liters to 0.5 liter by ultrafiltration using 10 kDa cut-off membrane. Then the ultrafiltrated concentrate was lyophilized and stored for tests.

The following #-s of cellulase dry preparations were used:

47.2001 - ATCC 34151 *Chrysosporium pannorum*,

47.2000 - ATCC 24782 *Chrysosporium pruinosum*,

47.1001 - VKMF-2875 *Chrysosporium keratinophilum*,

47.1000 - VKMF 2116 *Chrysosporium queenslaandicum.*

Protein content and activity fingerprints of these preparations are given in Table 4.

Example 9 -- Stone Wash Tests

A. Tests with 2-L special washing machine. This system assesses the stone wash performance characteristics related to abrasion and backstaining using only small amounts of enzyme.

Desizing. Forty pieces (30 g each, 25 x 20 cm) of denim fabric (roll) (1.2 kg) were desized in a household washer at 60°C for 20 minutes using a fabric:liquor ratio of 1:6 (7.2 L) and 0.5 g/L (3.6 g) Sandoclean PC liquid (nonionic washing and wetting agent on base of ethyoxylated fatty alcohols with an average of 6 moles of ethylene oxide, 1 g/L (7.2 g) Sirrix 2UD (acidic buffered sequestration) and 1 g/L (7.2 g) Bactosol TK liquid (high temperature stable alpha-amylase) at a pH of about 5 to 6. After 20 minutes, the liquor was drained and the pieces washed for 5 minutes with cold water (14 L) liquid ratio 1:10. The pieces were dried at 40°C and used as a stock of comparable samples for the determination of cellulase activity

The cellulase treatment of the garment pieces was carried out in a washing machine consisting of an inner drum of 29 cm diameter drum - 10.6 l total volume (drum rotates at 20 rpm - five turns left - five turns right). Each piece of fabric was sewn together with 4 rubber stoppers prior to the cellulase treatment to give a garment package that ensured that the mechanical effect occurred mainly on the darker outer side of the garment. Each drum was filled with one package and 10 additional rubber stoppers.

The general wash conditions were: 30 g desized denim jean fabric, cellulase in 0.02 M citrate buffer, 50°C, 60 minutes, garment:liquor ratio 1:4. After the cellulase treatment the package was washed with hot water (50 C) (garment:liquid ratio 1:20) for 5 minutes and dried for evaluation.

Application trials were conducted using various C1 cellulase preparations

For example, in ion exchange chromatography, it is possible to separate the cellulase components by eluting with a pH gradient, or a salt gradient, or both. Such separations can be done by those skilled in the art having the benefit of the teachings provided herein.

Once the individual enzymatic components of the cellulase composition are fractionalized and isolated the proteins may be partially sequenced or microsequenced to design synthetic DNA or probes to isolate the gene encoding the enzymatic proteins of interest. Generally the amino terminal sequence of the protein is determined by conventional protein sequencing methods or by automated sequence (Ausubel et al., (1987) in "Current Protocols in Molecular Biology", John Wiley and Sons, New York, New York). Alternatively, other regions of the protein may be sequenced in combination with chemical cleavage or enzymatic cleavage and protein separation techniques. (Ausubel et al., (1987) in "Current Protocols in Molecular Biology", John Wiley and Sons, New York, New York). One of skill in the art will understand that the synthetic DNA clones or probes can be used in routine cloning techniques to isolate the genes corresponding to the enzymes present in the neutral/alkaline cellulase compositions produced by *Chrysosporium*.

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It will be understood by one skilled in the art that nucleic and sequences obtained by this invention in the art may vary due to the degeneracy of the genetic code variations in the DNA sequence, but will still result in a DNA sequence capable of encoding the enzymatic components of the cellulase compositions. Such DNA sequences are therefore functionally equivalent to the nucleic acid sequences of the instant invention and are intended to be encompassed within the present invention. Also intended to be encompassed within this invention are nucleic acid sequences which are complementary to nucleic acid sequences capable of hybridizing to the disclosed nucleic acid sequence under a variety of conditions.

This invention further includes the protein or peptides or analogs thereof encoding the enzymes of the cellulase compositions of this invention and

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3. VKMF 2121	1.9	0	0.57	1.1	0.25	0.10	2.5	0.25	0.09
4. ATCC 24782	3.4	0.33	1.40	1.9	1.85	0.11	3.0	1.10	0.06
5. ATCC 34151	1.0	1.54	0.90	0.9	0.17	0.20	4.3	0.81	0.90
6. ATCC 44006	4.4	0.21	0.49	2.0	0.68	0.34	2.5	1.29	0.06
7. VKMF 2119	4.1	0	0.08	2.7	0.29	0.00	3.8	0.95	0.04
8. VKMF 2120	4.5	0	0.17	2.3	0.23	0.00	2.3	0.12	0.00
9. VKMF 2875	1.6	0	1.01	1.7	0.00	0.00	3.8	1.96	0.05
10. VKMF 2877	2.4	0	0.03	0.8	0.22	0.00	5.0	0.43	0.00
11. C1 (VKMF 3500D)	2.9	1.70	1.65	nt	nt	nt	0.1	0.89	0.80

RS = concentration of reducing sugars in the fermentation medium at the end of fermentation, g/L (Nelson-Somogyi method).
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Example 9 -- Stone Wash Tests

A. Tests with 2-L special washing machine. This system assesses the stone wash performance characteristics related to abrasion and backstaining using only small amounts of enzyme.

Desizing. Forty pieces (30 g each, 25 x 20 cm) of denim fabric (roll) (1.2 kg) were desized in a household washer at 60°C for 20 minutes using a fabric:liquor ratio of 1:6 (7.2 L) and 0.5 g/L (3.6 g) Sandoclean PC liquid (nonionic washing and wetting agent on base of ethyoxylated fatty alcohols with an average of 6 moles of ethylene oxide, 1 g/L (7.2 g) Sirrix 2UD (acidic buffered sequestration) and 1 g/L (7.2 g) Bactosol TK liquid (high temperature stable alpha-amylase) at a pH of about 5 to 6. After 20 minutes, the liquor was drained and the pieces washed for 5 minutes with cold water (14 L) liquid ratio 1:10. The pieces were dried at 40°C and used as a stock of comparable samples for the determination of cellulase activity

The cellulase treatment of the garment pieces was carried out in a washing machine consisting of an inner drum of 29 cm diameter drum - 10.6 l total volume (drum rotates at 20 rpm - five turns left - five turns right). Each piece of fabric was sewn together with 4 rubber stoppers prior to the cellulase treatment to give a garment package that ensured that the mechanical effect occurred mainly on the darker outer side of the garment. Each drum was filled with one package and 10 additional rubber stoppers.

The general wash conditions were: 30 g desized denim jean fabric, cellulase in 0.02 M citrate buffer, 50°C, 60 minutes, garment:liquor ratio 1:4. After the cellulase treatment the package was washed with hot water (50° C) (garment:liquid ratio 1:20) for 5 minutes and dried for evaluation.

Application trials were conducted using various C1 cellulase preparations

Claims

1. An isolated culture of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D.

2. A neutral and/or alkaline cellulase composition obtained by a method which comprises:

(a) growing a wild type or mutant *Chrysosporium* culture on a suitable medium; and

(b) isolating said neutral and/or alkaline cellulase composition from said *Chrysosporium* culture.

3. The cellulase composition of claim 2 wherein said activity is assayed by CMCase, RBBCMCase, Cellazyme, endoviscometric or filter paper activity assay.

4. The cellulase composition of claim 2 having maximal activity at a temperature from about 40°C to about 60°C, at a pH from about 5.0 to about 12.0 when assayed by an assay according to claim 3.

5. The cellulase composition of claim 2 having at least 50% of the optimal enzymatic activity using an assay according to claim 3 at about pH 6.0 to about pH 7.0 at about 40° to about 60°C using the same assay method.

6. The product, according to claim 2, wherein said *Chrysosporium* culture is *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium guenslandicum, or Chrysosporium tropicum.*

7. The product, according to claim 6, wherein said *Chrysosporium* culture is *Chrysosporium lucknowense.*

8. The product, according to claim 7, wherein said *Chrysosporium lucknowense* culture is *Chrysosporium lucknowense Garg 27K*, accession number VKM F-3500D.

9. The product, according to claim 2 wherein said *Chrysosporium* culture is a mutant strain of *Chrysosporium.*

10. The product, according to claim 9 where said culture is a mutant strain of *Chysosporium lucknowense Garg 27K.*

11. The product, according to claim 2, wherein said medium comprises inorganic salts, complex organic nutrient sources, or a combination thereof, and a carbon source.

12. A method for producing a neutral and/or alkaline cellulase composition, said method comprising

(a) growing a wild type or mutant *Chrysosporium* culture on a suitable medium; and

(b) isolating said neutral and/or alkaline cellulase composition from said *Chrysosporium* culture.

13. The method, according to claim 12, wherein said *Chrysosporium* culture is *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium guenslandicum, or Chrysosporium tropicum.*

14. The method, according to claim 13, wherein said *Chrysosporium* culture is *Chrysosporium lucknowense.*

15. The method, according to claim 14, wherein said *Chrysosporium lucknowense* culture is *Chrysosporium lucknowense Garg 27K.*

16. The method according to claim 12, wherein said *Chrysosporium* culture is a mutant strain of *Chrysosporium.*

17. The method according to claim 16 wherein said mutant strain is a mutant of *Chrysosporium lucknowense Garg 27K.*

18. The method, according to claim 12, wherein said medium comprises inorganic salts, an organic nutrient source, or a combination thereof, and a carbon source.

19. A laundry detergent or fabric softening composition comprising a neutral and/or alkaline cellulase composition according to claim 2.

20. A method of stonewashing jeans, said method comprising treating said

jeans with the neutral and/or alkaline cellulase composition of claim 2.

21. A method of biopolishing, defribillating, bleaching, dying, color brightening or desizing textiles comprising treating said textiles with neutral and/or alkaline cellulase composition of claim 2.

22. A method of deinking or biobleaching paper or pulp said method comprising treating said paper or pulp with said neutral and/or alkaline cellulase composition of claim 2.

23. A method for enhancing the softness or feel of cellulose or cotton containing fabrics comprising treating said fabric with a neutral and/or alkaline cellulase composition according to claim 2.

24. Purified or partially purified polypeptide or protein fractions having neutral or alkaline cellulase activity and obtained from neutral and/or alkaline cellulase compositions according to claim 2.

25. A nucleic acid sequence encoding a peptide or protein comprising the neutral and/or alkaline cellulase compositions according to claim 2.

26. A method for generating mutants of *Chrysosporium* producing cellulase composition, having enhanced activity at neutral and/or alkaline pH's comprising

(a) mutating spores of *Chrysosporium*;

(b) culturing said spores from step (a); and

(c) screening said cultures for enhanced levels of neutral and/or alkaline cellulase activity.

27. The method of claim 26 wherein said mutating step comprises exposing said spores of step (a), to ultraviolet light or chemicals.

28. The method of claim 26 wherein said chemical is nitrous acid, N-methyl-N'-nitro-N-nitrosoguanidine, or 4-nitroquinolone-N-oxide.

29. A neutral and/or alkaline cellulase composition produced by said mutants according to claim 26.

30. A mutant of *Chrysosporium* obtained by the method of claim 26.

31. Substantially purified and isolated peptides or proteins fractions obtained from the neutral and/or alkaline cellulase composition according to claim 29.

32. Isolated nucleic acid sequences encoding the peptides or proteins of claim 31.

33. A method of isolating genes encoding cellulase enzymes of *Chrysosporium* comprising:

a) isolating a protein from a neutral and/or alkaline cellulase composition produced by a wild type or mutant *Chrysosporium*;

b) sequencing all or part of said protein isolated in step (a)

c) producing a nucleic acid probe derived from said sequence of step (b);

d) screening a wild type or mutant *Chrysosporium* library with said nucleic acid probe of step (c);

e) isolating a nucleic acid sequence recognized by said probe; and

f) sequencing said nucleic acid sequence isolated in step (c).

34. The nucleic acid sequences obtained from the method of claim 33.

35. A recombinant expression vector comprising a nucleic acid sequence of claims 32 or 34.

36. The recombinant expression vector of claim 35 wherein said expression vector is a eukaryotic or prokaryotic expression vector.

37. A host cell containing the recombinant expression vector of claim 35.

38. The host cell of claim 37 wherein said host cell is selected from the group consisting of yeast cells, fungal cells, plant cells or seeds, and bacterial cells.

39. The fungal cells of claim 38 wherein said cells are selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Penicillium*, *Chrysosporium*, or *Neurospora*.

40. A composition comprising a solid or liquid medium containing an enzyme isolated or obtained from *Chrysosporium*, said enzyme having cellulase activity when suspended in an aqueous medium of neutral or alkaline pH.

41. The composition according to claim 40 wherein the aqueous medium has a pH of at least 5.5.

42. The composition according to claim 40 wherein the aqueous medium has a pH between at least 5.5 and about 12.

43. The composition according to claim 40 wherein the cellulase activity is assayed by CMCase, RBBCMCase, cellazyme, endoviscometric or filter paper activity assay.

44. The composition according to claim 40 wherein the enzyme has maximal cellulase activity when the pH of the aqueous medium is between about 5.5 and about 7.5 and the temperature of said medium is between about 40°C and about 60°C and when assayed using an assay method of claim 43.

45. The composition according to claim 40 wherein the enzyme has maximal cellulase activity at a pH of less than about 5.5 using an assay method according to claim 43 but still exhibits at least 50% of maximal cellulase activity with the same assay method when the aqueous medium has pH between about 6.0 to about 7.0 and temperature between about 40°C and about 60°C.

46. The composition according to claim 40 wherein the solid medium is in the form of a powder, granule, or freeze-dried form.

47. The composition according to claim 40 wherein the composition also contains a detergent ingredient and/or softening agent.

48. The composition according to claim 40 wherein the composition also contains at least one other enzyme.

49. The composition according to claim 40 wherein the composition also contains a dye substance.

50. The composition according to claim 40 wherein the aqueous medium contains components sufficient to facilitate the wash performance of the cellulase activity.

51. The composition according to claim 40 wherein said *Chrysosporium* is of a species selected from the group consisting of *Chrysosporium lucknowense, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium keratinophilum, Chrysosporium lobatum, Chrysosporium merdarium, Chrysosporium guenslandicum, and Chrysosporium tropicum.*

52. The composition according to claim 40 wherein the *Chrysosporium* is *Chrysosporium lucknowense.*

53. The composition according to claim 40 wherein the *Chrysosporium* is *Chrysosporium lucknowense Garg 27K.*

54. A substantially purified and isolated protein fraction, having cellulase activity at neutral and/or alkaline pH, and having at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0 as measured by an assay method according to claim 43, and obtained from the *Chrysosporium* composition according to claim 2.

55. An endoglucanase obtained from a fraction according to claim 54 and having molecular weight of about 25 kD and pI of about 4.

56. An endoglucanase obtained from a fraction according to claim 54, and having molecular weight of about 70 kD and pI of about 4.

57. An endoglucanase obtained from a fraction according to claim 54, and having molecular weight of about 60 kD and pI of about 3.

58. An endoglucanase obtained from a fraction according to claim 54 and having molecular weight of about 43 kD and pI of about 3.

59. A cellobiohydrolase obtained from a fraction according to claim 54 and having a molecular weight of about 60 kD and pI of about 4.

60. A substantially purified and isolated neutral and/or alkaline cellulase enzyme having a pI of between about 3 and about 5.5.

61. The cellulase according to claim 60 wherein said cellulase shows either endoglucanase or cellobiohydrolase activity.

62. The cellulase according to claim 60 wherein said cellulase retains at least 50% of its maximal cellulase activity at a pH between about 6.0 and about

7.0 as measured by an assay method according to claim 43.

63. The protein fraction according to claim 54 wherein the cellulase activity is useful in stonewashing procedures at pH between about 5.0 and about 7.0.

64. An endoglucanase obtained from the fraction according to claim 63 and having molecular weight of about 25 kD.

65. An endoglucanase obtained from the fraction according to claim 63 and having molecular weight of about 70 kD.

66. An endoglucanase obtained from the fraction according to claim 63 and having molecular weight of about 43 kD.

67. A method of stonewashing denim and other fabrics, comprising treating said fabric with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0 and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from the neutral and/or alkaline cellulase activity according to claim 54.

68. The method according to claim 67 wherein one of the cellulase enzymes is the endoglucanase of claim 55.

69. The method according to claim 67 wherein one of the cellulase enzymes is the endoglucanase of claim 56.

70. The method according to claim 67 wherein one of the cellulase enzymes is the endoglucanase of claim 57.

71. The method according to claim 67 wherein the cellulase enzyme is the endoglucanase of claim 58.

72. The method according to claim 67 wherein the cellulase enzyme is the cellobiohydrolase of claim 59.

73. A detergent composition containing one or more purified enzymes isolated from the protein fraction according to claim 54.

74. A fabric softening composition containing one or more purified enzymes obtained from the protein fraction according to claim 54.

75. A method of color brightening fabrics, comprising treating said fabric with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0 and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from a neutral and/or alkaline cellulase activity according to claim 54.

76. A method of softening fabrics, comprising treating said fabric with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0 and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from a neutral and/or alkaline cellulase activity according to claim 54.

77. The method according to claim 76, wherein the fabric is a cellulose of cotton containing fabric.

78. A method of depilling fabrics, comprising treating said fabric with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0 and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from a neutral and/or alkaline cellulase activity according to claim 54.

79. A method of biopolishing fabrics, comprising treating said fabric with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0 and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from a neutral and/or alkaline cellulase activity according to claim 54.

80. A method of deinking or biobleaching paper or pulp, comprising treating said paper or pulp with at least one substantially purified and isolated cellulase enzyme having cellulase activity at neutral and/or alkaline pH and retaining at least 50% of maximal cellulase activity at a pH of between about 6.0

and about 7.0 as measured by an assay method according to claim 43 and where said cellulase was obtained from a neutral and/or alkaline cellulase activity according to claim 54.